The effects of cytochalasin D on the bile canaliculi formed between cultured rat hepatocytes

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Bile canaliculi are the structure delivering bile secreted by hepatocytes into the bile passage. It forms a secure environment isolated by junctional complexes composed of tight junctions and desmosomes. Bile secretion is mainly controlled by the cytoskeletal elements, mainly of actin in the microvilli and pericanalicular web. Cytokeratin intermediate filaments, function of which is not illucidated fully yet, are scattered in the pericanalicular ectoplasm, and some microtubules are found near bile canaliculi.

Most of studies on the bile secretion are done *in vivo* situations, however, to control the various parameters some *in vitro* culture system seem to be more useful. To set up a *in vitro* experimental system for the study on bile secretion and hepatocyte functions, the investigators tried to isolate hepatocytes with an enzymatic method using a mixture of collagenase and hyaluronidase from normal Sprague-Dawley rat liver and observed. Special focus was given to the bile canaliculi and actin cytoskeleton.

Isolated hepatocytes were round and formed cords in culture. Microvilli covered the whole surface of cultured hepatocytes. Bile canaliculi were formed between hepatocytes and were characterized by the presence of microvilli of various length and shape mainly arising from small surface mounds. Actin filament core in the microvilli and pericanalicular actin web were incomplete. Ruthenium red staining reveals cell surface mainly in the intercellular space. Structures in the bile canaliculi were not stained except for a few cases, however, ruthenium red often labels intracanalicular structures. HRP cytochemisty revealed the intercellular space, bile canaliculi and some intracelluar tubular and vesicular structures.

After cytochalasin D treatment, cultured hepatocytes were round but the surface were irregular with surface blebs, folds and grooves. Microvilli on the surface were scarce. Bile canaliculi were markedly dilated with often detached junctional complexes. Bile canaliculi lack microvilli almost completely and extended into pericanalicular cytoplasm showing complex vacuolar and tubular structures by routine and ruthenium red-stained transmission electron mciroscopy. In the pericanalicular actin web, intermediate filaments were hardly identified. Subsurface actin filaments were scattered scarcely under the cell membranes. HRP reaction

products fail to label bile canaliculi, however vesiculotubular structures similar to those observed in normal control group were present.

These results suggest that hepatocytes isolated from rats can survive and form bile canaliculi in culture and the actin filaments are involved in the formation and/or maintenance of bile canaliculi.

Figs. Normal bile canaliculi formed between cultured rat hepatocytes. Note the microvilli and junctional complexes. Actin filament web is absent (upper). Ruthenium red stains hepatocyte surface of the intercellular space but not in the bile canaliculus (lower).